CHROMOSOME STUDIES IN KUHNIINAE (EUPATORIEAE). I. BRICKELLIA

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(*Continued from p. 288*)

Seeds of the northerly species *B. Greenei* Gray, which has a limited distribution in northern California and Oregon, have been recently received. In figure 41 of a large periblem cell, where the chromosomes overlap hardly at all, their sizes as well as the spaces between them indicate the absence of any long ones. At least four central ones are medium in length and the remainder are short, two pairs at least short short. Though it was out of focus for the photograph of the inner tip of the medium chromosome seen at center bottom, there is a small satellite. In another cell, there were two such chromosomes (fig. 46), outer, lower and upper left. By the presence of this pair, as well as the lack of any long chromosomes, the karyotype of this species more nearly resembles that of *B. incana* (cf. figs. 47, 48) than any other. By a comparison with those of figures 1 to 30 it is apparent that by the lengths of the chromosomes it would have to be placed within the second row before those of the *Microphyllae*, which have been referred to as a general karyotype.

Of the two species, *B. oblongifolia* var. *linifolia* and *B. macromera*, not enough dividing cells were seen to permit karyotypic analysis or to select adequate ones for photography. Nevertheless, besides enabling accurate counts in both, the figure of *B. oblongifolia* justifies the statement that its karyotype is more similar to those with longer chromosomes than to *B. incana* with a complement of short units. *B. macromera* appeared to be more like *B. californica.*

SECTION IX. MACROBRICKELLIA.

This section represents the ultimate size of the head in the genus, as many as one hundred and thirty-eight florets having been counted in a head of the specimen received. *B. monocephala*, in the first preparations of inactive tissues, gave evidence of comparatively large nuclei, being almost twice the size of those in *B. diffusa*, which was being studied at the same time. When seen, this karyotype clearly consisted of longer chromosomes than any other examined (fig. 30). The two shortest pairs were well equal to what were classed as medium ones elsewhere and proportionately, the medium and long chromosomes were longer than in others. In the figure are seen across the top, three of the long submedian class with the shorter arm of one a little blurred by the overlying long median one. The two on either end are a pair, while the mate to the middle chromosome, clearly in focus at center right, emphasizes that these two pairs of heterobrachial chromosomes show different proportions in length of arms. Though one would hardly call this latter pair subterminally constricted, it is quite possible that reduction in total length of this chromosome would result in about the equivalent of an Lst of other karyotypes. In the row of four chromosomes of medium length at center left, can be seen in succession 1 Mm, 1 Mst, 1 Msm, 1 Mst, respectively. Completing all the classes of this karyotype, the shortest length is seen in one just next to the long submedian one at the right. With a little attention, each of the classes can be seen in this cell without resorting to stylized idiograms.

SUMMARY ON KARYOTYPES

In the thirty-three perennial herbs and shrubs carefully examined, there is one chromosomal complement, or a complement so closely approaching it that it cannot be distinguished, common to fifteen species. This has been called the general karyotype since it involves the greatest number of species. It is represented, approximately in the order of the lengths of the chromosomes, as 1 Lsm, 1 Lm, 1 Lst, 1 Msm, 2 Mm, 1 Mst, 2 Sm. Since so many species did not differ recognizably in the morphology of their chromosomes, it is concluded that speciation is in a large measure due to genic changes.

Quite distinctive karyotypes were found, however, in:

(1) The predominantly short chromosomes of *B. laciniata*, *B. desertorum*, *B. californica*, and the nearly as short ones of *B. veronicaefolia*.

(2) Similar to (1), the almost indistinguishable karyotypes of *B. Rusbyi* and *B. floribunda*.

(3) The distinctive complement of short chromosomes, with one pair showing satellites, of *B. incana* and *B. Greenei*.

(4) *B. Coulteri*, similar to the general complement, but with one pair having satellites.

(5) The singular complement of entirely long and medium chromosomes, longer than in other species, and lacking any short ones, of *B. monocephala*.

(6) *B. grandiflora*, with long and medium chromosomes only.

(7) The fewer short (one pair rather than two) in the complement of predominantly longer ones, of *B. Wislizeni*, *B. peninsularis*, *B. argyrolepis*, *B. adenocarpa*, *B. pacayensis*, and also in *B. betonicaefolia* and *B. amplexicaulis*.

A little less striking but real, the tendency of all-around shorter chromosomes in *B. dentata* of the *Parvulae*.

There are included in the first three categories (involving eight species), evidences of a smaller amount of chromatin in the much shorter chromosomes, than in what is here referred to as the general complement. In the last three categories (nine species), the evidence for longer and fewer short chromosomes amounts to a greater total of chromatin material than in that of the general karyotype. The additive effect in these two groupings increases the difference in the extremes of karyotypes found within the perennials.

The karyotype of only one annual was seen and that distinctly falls in with those of lesser chromatin content having chromosomes of reduced length.

Meiotic Studies

OBSERVATIONS

It has been possible thus far to see the meiotic chromosomes in twenty-six accessions[8] of twenty-one species. Among these, there is a representation of each section and subsection of which the karyotype had been studied, except for the first and last sections, and of subsection *Brachiatae* to which *B. Coulteri* belongs. *B. diffusa* and *B. monocephala* would have made interesting additions to confirm the differences in their chromosome sizes, seen in somatic figures. *B. scoparia*, subsection *Clavigera*, was additional, as in it mitosis has not been seen.

[8] Material of additional accessions of some species has been collected but the suitable meiotic stages have not as yet been found in them.

Fixed preparations, in which the size of units could be suitably compared because of the uniformity with which the field material was treated, showed quite similar plates of 9 chromosomes. In those of *B. veronicaefolia* varieties *veronicaefolia* and *senilis*, the first and second metaphase plates appeared smaller than others, but due to unfavorable staining were not photographed. They were not so large as those of *B. scoparia* (fig. 50) which are perhaps a trifle smaller than those of *B. verbenacea* and *B. glomerata* (figs. 51, 53), both species having the general karyotype. None of the other species having complements of the smaller chromosomes were collected in Mexico and so are not included. However, a cell of *B. californica I*, from an acetocarmine smear of pollen mother cells, is shown in figure 56. Although by that technique, chromosomes are invariably swollen so that they appear larger than in fixed preparations, these are certainly no larger than those of *B. scoparia.* The other haploid counts had been obtained by means of smears, but slides of only a few were kept.

As the number of florets per head varies from eight (e. g., in *B. scoparia*) upwards, there is frequently an unusual variety of both first and second[9] division stages even in sections of heads that have been cut at time of fixation to allow better infiltration of fluids. Successful fixation, without shrinkage, resulted in heads numbering up to twenty-seven florets, which had been left uncut. Meiosis was found to proceed regularly except in a single collection of each of two species, *B. reticulata* and *B. pendula;* in one of two collections examined of a third species, *B. glomerata;* and in one of three collections of a fourth, *B. adenocarpa.* In preparations of *B. glomerata I*, very regular metaphase plates were seen (fig. 53). In contrast, such could not be found in *B. glomerata V*, for at best one or two units were on a different focal level. In lateral anaphases and telophases, chromatin bridges were at once evident (fig. 54, 55). In the latter figure, a tiny unit was divided late and probably represents the fragment of an inversion. In *B. adenocarpa Ia*, there were found in anthers of the same floret, regular metaphase plates as in figure 59, and also two cells in early telophase, each with two very similar belated chromosomes approaching the

[9] Hereafter first and second division stages will be referred to as I and II respectively.

[10] In conversation with the late Prof. Conzatti, he stated this species was very difficult to find.

poles. In one of the cells (fig. 60), it can be seen that the laggards are approximately the same size as the chromosomes which had already reached the poles. Attached to the one at the bottom is a small fragment, probably again that of an inversion. Careful examination of the sections showed many regular I and II metaphase and telophase stages but occasionally there was evidence of one or two bivalents splitting earlier on the plate.

Preparations of *B. reticulata* offered a strong contrast to those of closely related *B. oliganthes* and *B. verbenacea* (fig. 51) the materials of which had also been collected in Mexico. Meiosis proceeded regularly in the two latter, and in *B. venosa* from Arizona in addition, while in *B. reticulata* all plates of I metaphase were slightly irregular. When seen laterally all of the chromosomes but one (fig. 52) or two were in a neat median line as though one or two bivalents had not been organized in the plate. In *B. pendula III*, the same kinds of disturbances were found; one outlying unit on lateral metaphases (fig. 58), less frequently two, and occasional telophases showing division of lagging units. In one cell, each of two univalents was dividing evenly, one on mid-spindle and the other close to one pole (fig. 57).

DISCUSSION

Just how abundantly such irregularities occur in this genus is still a question. One of the four referred to here, was collected in the field in Guatemala and the other three, in Mexico on three different dates in as many different states. The incidence among the more southern species seems of a rather high proportion, 4 out of 13, when compared to smears from greenhouse plants of an equal number of other species, where no irregularities were noticed.

Irregularities in meiosis of the kinds described are usually associated with structural hybridity, indicating that inversions have taken place. They are well known in interspecific hybrids and are also found in a number of species (Darlington, 1937, and Dobzhansky, 1941). Various species of *Paeonia* (Dark, 1936; Stebbins, 1938) are especially remarkable in that all individuals studied gave evidence of one or more inversions. The species of *Brickellia* affected, belong principally to the subsection *Coleosanthus,* although one is of the *Reticulatae.* The observations bring up very interesting points in relation to these species.

Discussion of *B. reticulata* (Gaiser, 1952) explains the doubt concerning this entity. Both Gray (1852) and Robinson (1917) had suggested that it might not be distinct from *B. oliganthes*. In this author's collections, from two different localities in the adjoining states of Michoacán and Jalisco specimens were found which come within the descriptions of *B. oliganthes* and *B. verbenacea* respectively, and from Morelos, this one plant of *B. reticulata*. They are clearly closely related, but cytologically the latter has been found to differ from the other two. If the meiotic irregularities seen are merely indicative of structural changes, such as inversions, it is generally considered that such have no effect on the phenotype but to alter gene linkage and introduce sterility (Gates, 1951). Although seeds were obtained from the first two specimens, it is not known whether this single plant of *B. reticulata* would have been fertile since it was in an early flowering stage. By the tomentose stems with numerous almost entire leaves it was recognized as differing from the other two species, and strongly resembling the figure of a single plant of Haenke's, which DeCandolle described as *B. reticulata*. Like it, the plant was robust and the inflorescence was large, characteristics that might be indicative of hybrid vigor. It may be that the chromosome behavior is the result of two not quite compatible genomes and that this plant represents some mid-form between a species with the somewhat serrate leaves, as seen in *B. oliganthes*, and another even less closely related species. At least three other species were collected within a stretch of seven kilometers on the same road from Cuernavaca to Jautepec, *B. glomerata III*, *B. paniculata*, and *B. scoparia*. Of these, the former was very abundant and grew close by the plant of *B. reticulata*. In material collected from *B. glomerata III*, the pollen had already developed and the grains were regular in form.

Of *B. glomerata*, it was possible to study meiosis in materials of the two accessions, I and V, collected respectively in Cuernavaca and from near Taxco. The first accession which had normal divisions with regular plates, had been noticed to vary in height and size of leaf from *B. glomerata II*, which was also collected along the river bank in Cuernavaca. From an examination of the Taxco specimen, collected in early flowering condition from a high exposed and sunny rock, the characters of leaves and heads seemed to come within the description of the species. This was also true of specimens from three other shrubs, taken near by, which were not in such advanced conditions, but varied considerably in height. In that region of Taxco, a sharp lookout was kept for the closely related species *B. hebecarpa* (DC.) Gray, from which it had been separated and which had been reported from Puenta de Ixtla, less than 50 kms. distant. Leaves which are basally acute rather than rounded, and heads pedicellate rather than glomerate, should distinguish the two species. While some of the material of *B. glomerata* had leaves that bore a resemblance to that species, none was found in this early stage with other than sessile heads. One is led to wonder if, with many more collections, these characters might fail as clear cut specific delimitations.

The accession of *B. pendula III* with lagging bivalents in meiosis, was found in Puebla, on the route from Jalapa to Puebla, on open scantily wooded slopes intermingled with shrubs of *B. veronicaefolia*. At the time of collection it was left unnamed. In part this can be explained by the uncertainty of distinguishing the species *B. secundiflora* var. *secundiflora* and *B. pendula* in the field. It is similar to two other collections made in Mexico, D. F. and Puebla, respectively. In comparison with specimens in the herbarium, this collection resembles *B. pendula* in its oblong-lanceolate leaves and the presence of reddish stipitate glands on the pedicels and phyllaries. It does have more flowers per head than is given by Robinson, 19 rather than ca. 12. In this it is more nearly like *B. secundiflora* with ca. 20 flowers.

The material of *B. adenocarpa Ia*, generally characterized by very normal divisions, showed in the anthers of one floret, chromosome bridges and regular first metaphase plates. The specimens of this accession were identified as variety *glandulipes*, having some glandular as well as some non-glandular trichomes on the pedicels and phyllaries. A few plants were found with first flowers which were yellow, establishing the species, which by herbage alone might have been confused with *B. paniculata*, of rosy pink flowers. *B. adenocarpa* var. *adenocarpa*, which lacks the glandular trichomes and has soft hairs instead, was not found along with it at this station. However, it is known to occur in the vicinity of Antigua, a little of it having been sent in dried condition, from the region where material of the fifth accession was taken. However, as there was no cytological fixation of it, there has been no examination of the variety distinctly lacking glandular trichomes.

All four species, in which the irregularities have been found, certainly raise critical questions. Is *B. reticulata* separated by significant isolation barriers from *B. oliganthes?* The same question arises concerning *B. glomerata V* in relation to *B. hebecarpa. B. pendula III* did not completely satisfy the species-description, comparing favorably with the closely allied *B. secundiflora* in number of florets per head. *B. adenocarpa* var. *glandulipes Ia* represents a transition between non-glandular and glandular pubescence. Since the same intermediacy occurs in two other species, *B. secundiflora* and *B. Palmeri*, where the same intergrading pairs were made varieties, this may be of less significance.

Stebbins has observed in species of *Paeonia* that, in those including a number of geographical races, the evidence for inversions was greater than in more uniform species. Certainly such a species as *B. scoparia*, studied from the same kind of preparations and regular in meiosis, is a very uniform species as found by the writer in Oaxaca, Michoacán, and Morelos. Once seen, *B. veronicaefolia*, although divided into four varieties by its pubescence, would always be recognized. In two of these varieties examined meiotically, it was perfectly regular. Looking over the list studied by smears from *B. microphylla* to *B. grandiflora*, few if any would be called large polymorphic species.

The greatest trouble seems to lie in subsection *Coleosanthus*, which with 22 species, is the largest. If the two species, *B. Rusbyi* and *B. floribunda*, from the New Mexico and Arizona region, are excepted, all the rest are fairly close Mexican and Guatemalan congeners. Without questioning the equivalence of species or races in different genera, it may be that this group is in a great state of flux, with more variations arising.

That there may be intermediates among a group of such closely related species, was recognized by Robinson (p. 83). A note after *B. secundiflora* refers to one specimen which he considered intermediate between that species and *tomentella*. It may be significant, that in collections concerning which there was no doubt as to identity, as the broader-leaved variety *nepetaefolia* of *B. secundiflora*, with strictly non-glandular trichomes, and *B. tomentella*, from the entrance to the valley of Orizaba, no disturbances of meiosis were found.

Trichomes

A study of leaves of the species of *Brickellia* which had been examined karyologically was begun to see if there were any correlations between the kinds of trichomes and their types of chromosomal complements. Since there was a splendid representation of this genus in the Gray Herbarium, a similar examination of most of the species was undertaken in case they might be of any further aid in taxonomic classification. Thus leaves were cleared of all but six species which were only scantily represented. As far as possible, leaves were chosen from annotated sheets mentioned in the monograph so that those studied would be of specimens close to the types.

At the outset, absence or presence of trichomes is not considered significant. From the experimental studies of Clausen, Keck and Hiesey (1940) on other genera of the *Compositae*, it was established that the amount of pubescence was one of the most easily modified characteristics in transplant experiments, but the type altered hardly at all. This suggests, in some genera perhaps, a promising use of the nature of the trichome in distinction of species.

The trichomes found in the majority of the species were multicellular and unseriate, the first class given by Metcalfe and Chalk (1950, p. 783) as common in the *Compositae*. The length of the cells in these may vary. When they are short and rather block-like, as in *B. betonicaefolia* (fig. 61), trichomes have here been called attenuate. A few very similar trichomes were found along the veins of *B. amplexicaulis*, the closely related species, which has a great predominance of glandular trichomes. The side walls of the attenuate type were generally very straight due to the greater support of the cross walls, as in the rungs of a ladder. In contrast, those with more distant septations and longer cells frequently had a slightly wavy outline due to a little narrowing in the middle diameter of the cells, which might have partly resulted from the treatment. This type looked rather "icicle-like" and will be termed long acuminate (*B. paniculata*, fig. 62), in contrast to attenuate. But the two are not sharply limited, *B. secundiflora* seeming to be intermediate between them. Trichomes of these two types were but one cell in diameter throughout their entire length, including the stalk cell. The latter was usually large and undifferentiated, frequently covering parts of more than one epidermal cell. Of course the total length varied considerably on any leaf, but on leaves of species described by Robinson as puberulent, the general length was short in comparison with the longer trichomes of the pubescent species. Such shorter trichomes were always more nearly similar and more difficult to place in either one or the other of these two classes. Illustrations are included of one of annual *B. diffusa* (fig. 63) where the leaf was described as sparsely puberulent or glabrous and of *B. monocephala* (fig. 64) similarly called puberulent. There were also variations in the thickness of the wall which helped to account for the firmness of those on so-called scabroid leaves. The cells of these were usually isodiametric and sometimes built on a base of several cells (*B. scabra*, fig. 65).

Of course on some leaves the indumentum was of erect or unbent trichomes, whereas others were appressed in varying degrees, and some of the longer ones were even somewhat coiled. Generally this did not alter the type. However, little variations were noted affecting the terminal cell or cells which appeared to be somewhat transitional to the next form to be described. *B. Palmeri*, of which a long and a short trichome were drawn *in situ* (fig. 66 and 66a), serves as an example, as might also *B. glomerata*. Similarly throughout the *Reticulatae*, a long acuminate type with a tendency towards a cap cell, is found consistently, although there are variations in the length and diameter of the trichome in the seven species (fig. 67, *B. reticulata*).

The most extreme form with terminal differentiation, represented in *B. veronicaefolia*, had the appearance of a terminal cap cell and is here referred to as "towards cap." Although not nearly as distinctive as those of Guayule shown by Rollins (1944), they have a definite tendency in that direction. Fig. 68 represents three trichomes drawn *in situ* from the surface of a leaf of *B. veronicaefolia* var. *veronicaefolia* showing that even in the shortest ones, whether erect or bent, the terminal cells are differentiated. This variety was described as very short pubescent. Furthermore on the leaf of variety *senilis* of this species, which was described as tomentose, the trichomes, although much longer, show a similar tendency towards cap cell (fig. 69). In this type the long side walls appear less strict due to a little more protruding in the proximal cell width.

What appears to be a modification from the attenuate type, has distinctly rounded rather than straight side walls, which give a bead-like appearance (*B. californica*, fig. 70). Almost always the longer hairs of this type were two cells wide at the base and sometimes through the proximal and antiproximal cells as well. This has been called moniliform here, which implies a more restricted use of the term than in Robinson's treatment where it applied also to *B. veronicaefolia* var. *senilis* described above. While one hesitates to classify shorter hairs as of any particular type, in *B. Rusbyi* (fig. 71) there is a strong suggestion of the moniliform. The longer trichomes along the mid-rib of the leaf of this species definitely resemble *B. californica* in their double basal cells as well as general contour. The type seems distinctive even in the very short ones, occurring very sparsely on the almost glabrous leaves of *B. laciniata* (fig. 72). Comparisons with figures of the other short puberulous ones emphasize this.

The thin-walled, much appressed trichomes of *B. dentata* differ from the other types in tapering less from base to tip and having even the apical cells rounded. Also they are narrower at the cross walls rather than at mid-diameter of the cell. It was not easy to follow one throughout its entire length as many were broken in the preparation. Two parts have been drawn here, one of the basal and wider cells (fig. 73) and the other of longer ones, characteristic of the tip (fig. 73a). No trichomes were found extending from the margin of this leaf. In no other species studied karyologically is there a match for this. Even among those in which the chromosomal complement has not been examined, no very close approximation was found. Leaves of two other species in the same subsection are either glabrous, as in *B. brachyphylla*, or scabroid puberulous as in *B. parvula* Gray. In the third species, *B. cylindracea* Gray, the trichomes are not extremely long either but are of the long acuminate type.

The most distinctive variation was the long thin-walled, almost entirely non-septate trichome which was found only in *B. incana.* Under the microscope, these seemed indefinite in length and there was difficulty finding the tip and base on the same one. Thus a comparatively short one, which came within the microscopic field, was drawn (fig. 74). While it shows one cross-wall, this was seen infrequently. In the majority none at all were found. A very rare instance of branching above the cross wall of the single stalk cell is represented in figure 74a. These long interwoven hairs easily form the matted condition for which the species is named. On the other hand, the "woolly" appearance for which DeCandolle named *B. lanata,* consists of very long hairs of the long acuminate type (fig. 75). Comparison of the trichomes of these two species which rank among the longest found in the genus, show those of *incana* to be exceptional not only in length and almost complete lack of septation, but also in the slight indication of branching.

In the section on the *Compositae,* Solereder (1899) described a kind of non-glandular trichome, consisting of a uniseriate pedestal with a long whip-like terminal cell and included an illustration (Fig. 103B) from Vesque (1885 Pl. 10 Fig. 5) for *Cirsium lanceolatum* Scop. In the addenda (Solereder 1908, p. 954), in a longer list of genera to which this type is said to be common, *Brickellia* is included without reference to any species. Metcalfe and Chalk *l. c.* referring to the same figure, give this as their second class of non-glandular trichomes in the *Compositae.* In our studies of *Brickellia,* the trichomes of *B. incana* would come nearest to qualifying for this type. It is, however, more comparable to figure 6, Pl. 10 (Vesque, *l. c.*) of *Antennaria plantaginea,* or figures 17 and 18 by Volkens (1887) for *Echinops spinosus* and *Atractylis flava,* because of the shorter stalk. Nevertheless it differs in the occasional septation and branching.

Of all the species, the only one other found to have branching trichomes was *B. Nevinii.* The trichomes there are septate (fig. 76), consisting of long cells similar to but slenderer than those of *B. lanata.* This species is outstanding in its variety of simple and branched trichomes. Sometimes where the stalk cell can be seen it is found definitely to give rise to several filaments. At other times this branching happens more distally. In this respect it differs from those of other species of the subsection. As stated above, Robinson referred to these four otherwise closely related species as varying markedly in their pubescence. *B. microphylla* has been found to have the glandular biseriate type of trichome (see fig. 78) common to a great many species of the genus. The trichomes of *B. scabra* (see fig. 65) are really very thick-walled attenuate forms and in *B. Watsoni* Robinson are found the long acuminate forms figured for *B. lanata* (see fig. 75). Actually when examined, it is seen that these two have been considered as modifications of the uniseriate attenuate form. From these *B. Nevinii* differs most because it branches.

In addition to the above kinds, glandular trichomes have been found in this genus. In some species, such as *B. Wislizeni*, the pubescence of the leaves consisted almost entirely of trichomes of this type. Only a very few non-glandular trichomes were found on the midrib below, near the petiolar attachment and these were attenuate and similar to those seen in *B. betonicaefolia* and *B. amplexicaulis*. In *B. Wislizeni* and *B. Greenei* the glandular trichomes were the longest found in the genus (fig. 77). Each trichome consists of a stalk always two cells in diameter or exceptionally three in the very bottom row of some of the largest ones. Seen laterally, the actual gland at the top of the stalk consists of several successive pairs of flattened cells, eight to twelve in number. In outline this gland is somewhat globular, though shrunken by the treatment. Though examination of pedicels was not undertaken, the same kind was found on the phyllaries when sectioning flower material of *B. pendula* (fig. 82). Since this species was described as stipitate glandular and was figured with head-like glands on both the pedicels and phyllaries (fig. 64, Robinson) as were also other species including *B. Wislizeni* and *B. floribunda*, it is evident that this type of glandular trichome is found frequently throughout the genus. Its prevalence or absence, especially on pedicels and phyllaries, also led to the separation of varieties within species, e. g., *B. adenocarpa* var. *glandulipes*.

In the examination of other species, various sizes of these glandular trichomes were found. On *B. frutescens* Gray they varied upwards in size from mere papillate outgrowths to lengths less than those found in *B. Wislizeni*. By microscopic examination, the shortest ones were found to consist of pairs of small closely appressed hemispherical cells, not very different from the crescentic cells of the stomata. Looking down upon the flat surface of a leaf preparation they are recognized as being raised ever so slightly above the rest of the epidermal cells by a little difference of focal depth (fig. 79). As the shortest are ever so little above the surface they could be seen laterally better along a midrib as consisting of only a few pairs of undifferentiated cells (fig. 80). In one of four tiers of cells, however, the lowermost appeared to have already become slightly differentiated as basal stalk cells (fig. 81). The cells increase in numbers with repeated divisions and the whole trichome becomes longer as in *B. microphylla* (fig. 78). The stalks are always two cells wide, except only in the occasional basal tier of the largest trichomes and the gland was never found to consist of more than two rows of cells. The whole trichome is composed of biseriate cells in contrast to that of all of the non-glandular trichomes.

It was further found from the examination of cleared leaves of the many species that pairs of small hemispherical cells appeared quite regularly somewhat sunken in the epidermis. Although they were usually larger and their mid-walls were more uniformly appressed, they did suggest stomata. When both were seen

Plate 1196

EXPLANATION OF FIGURES

Figs. 61–76. Types of trichomes. 61. *B. betonicaefolia* (attenuate). 62. *B. paniculata* (long acuminate). 63. *B. diffusa* and 64. *B. monocephala* (short attenuate). 65. *B. scabra* (scabroid). 66. *B. Palmeri* var. *amphothrix*, long, and 66a, short, drawn *in situ*, and 67. *B. reticulata* (long acuminate towards cap differentiation). 68. *B. veronicaefolia* var. *veronicaefolia*, three drawn *in situ* and 69. *B. veronicaefolia* var. *senilis* (towards cap). 70. *B. californica* (moniliform). 71. *B. Rusbyi* (moniliform). 72. *B. laciniata* (short moniliform). 73. *B. dentata*, basal and 73a, apical. 74. *B. incana* (unseptate) and 74a, stalk cell with two branches. 75. *B. lanata* (very long acuminate). 76. *B. Nevinii* (branched).

Figs. 77–81. Glandular trichomes, biseriate, on leaves. 77. *B. Wislizeni* (large). 78. *B. microphylla*. Figs. 79–81. *B. frutescens*. 79. Origin in slightly raised pair of cells. 80. Lateral view of three tiers of undifferentiated cells. 81. Lateral view of four tiers of cells with basal stalk cells differentiated. 82. On phyllary: *B. pendula*. Figs. 83, 84. Punctate conditions. 83. *B. monocephala* (lower epidermis). 84. *B. cuspidata*, upper and 84a, lower epidermis, between the same two veins respectively. Figs. 85, 86. In margin of leaf, *B. glutinosa*. Figs. 87, 88. glandular trichomes, uniseriate, on leaves. 87. *B. argyrolepis*. 88. *B. Coulteri*. All drawings were made from cleared leaves except for Fig. 82, which was from a fixed, sectioned preparation, by use of camera lucida, × 510, reproduced at 1275 ×.

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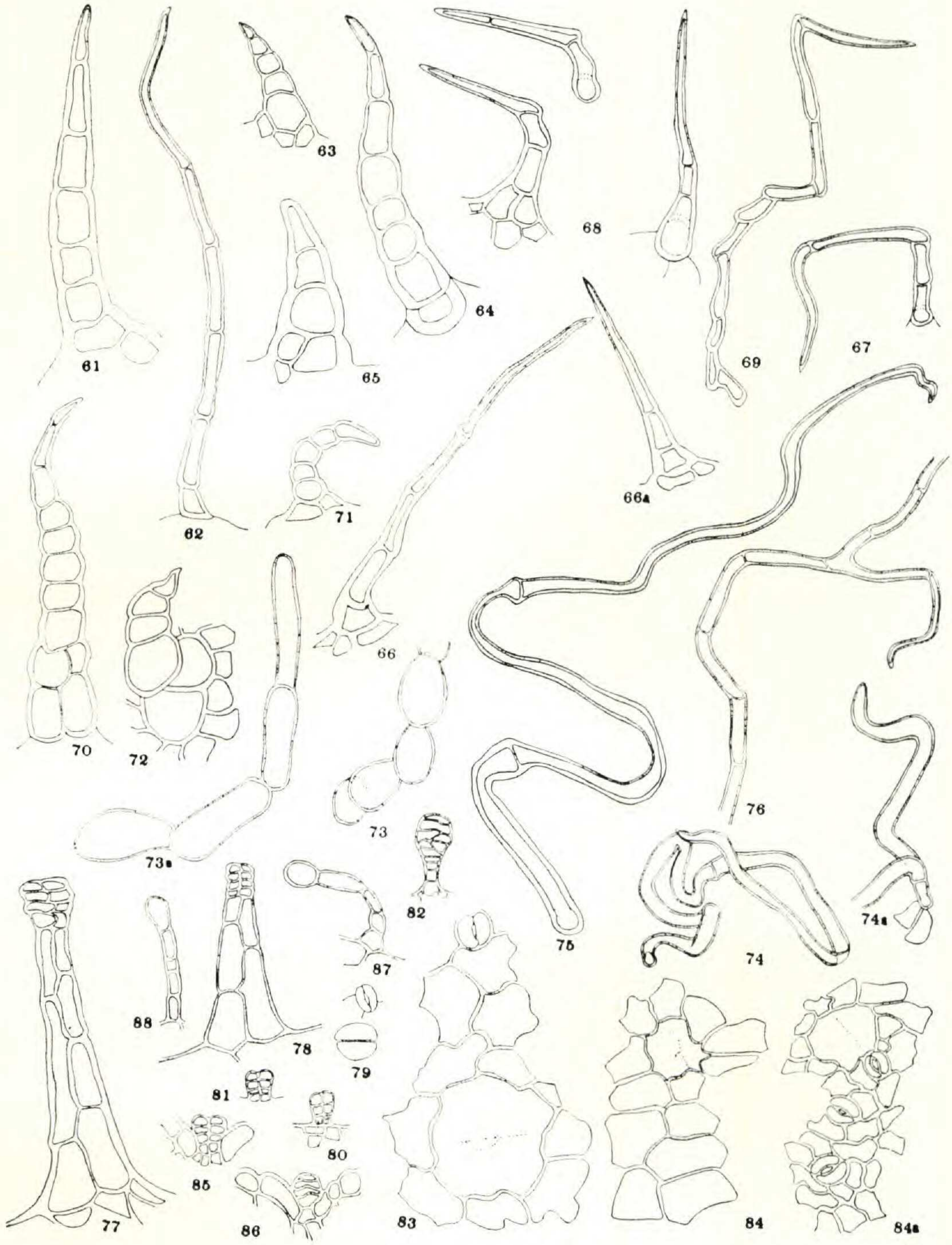

FIGURES 61–88

together the difference was clear for that particular species. However, they can be very confusing because: (a) sometimes they are much smaller than the guard cells of the stomata (e. g., *B. cardiophylla* Robinson, upper epidermis); (b) they may be very sparsely scattered in some species (e. g., *B. Palmeri*) as are also the stomata on the upper surface; (c) they may be so abundant that it almost seems that they are the stomata (e. g., *B. glutinosa* Gray); (d) the size of the epidermal cells of leaves varies from species to species and the guard cells with them; (e) in some species the pattern on the upper and lower surface of the same leaf is so different in the size of cells as to cause confusion (e. g., *B. cuspidata*); (f) they may occur on both surfaces or more abundantly on the lower one. The only safe way was to find a stoma of that leaf surface for comparison.

These paired cells belong to the sessile glands to which reference was made by Robinson in *B. pulcherrima* and *B. Palmeri*. Robinson also called them atomiferous glands in *B. cuspidata, B. peninsularis* and *B. cordifolia* Ell. and minute glands in *B. diffusa*. They are conspicuous to the examiner of a leaf when a droplet of secretion can be seen. If the cells have not yet become secretory, it is very doubtful whether they would be seen. Since the size of the leaf chosen for this study was more suitable if it could be placed on a microscope slide, small and perhaps young leaves were often taken from the herbarium sheets. It was possible to find the glands by microscopic examination in almost all the species. None were seen either microscopically or macroscopically in the leaves of the limited specimens available of *B. glabrata* (Rose) Robinson, *B. brachiata* Gray or *B. megalodonta* Greene, and they occurred sparsely in *B. cymulifera* Robinson. On *B. hastata* Benth. and *B. megaphylla* they occurred less scantily. These are the species of very restricted distribution of the *Brachiatae*. All the specimens of the related *B. Coulteri* in the Gray Herbarium were examined with a hand lens and on only five of twenty-four of them could glandular secretions be seen.

It was found that the punctate condition referred to in a number of species of this genus, is associated with the position of these glands. When preparations of leaves of *B. scoparia, B. hymenochlaena* and *B. monocephala* were examined under the microscope, the epidermal pattern was seen to be interrupted by what appear to be large "holes." Upon closer examination, in these can be found the same double cells, though usually on a lower level. No leaf sections were made to study the cellular details. However, they appeared to be somewhat funnel-shaped with a smaller inner diameter. Two oval bodies flattened against the adjoining mid-walls were usually distinguishable when, in the same preparation, no content was recognizable in the guard cells (*B. monocephala*, fig. 83). The two gland cells are bordered by a rather circular arrangement of smaller cells. Under the overlapping margins of the epidermal cells small foreign particles may collect. This emphasizes the slightly sunken nature of the gland. In other species described as minutely punctate or puncticulate (e. g., *B. dentata* and *B. macromera*) the gland cells, and consequently the "holes," are smaller. In *B. cuspidata* they are of intermediate size. This species serves well to illustrate the difference in size and distribution on the upper and lower epidermis of the same leaf (figs. 84 and 84a) which may account for Robinson's description of glandular atomiferous above and obscurely punctate below. It also may be possible that the secretions on the lower surface were not concurrently visible with those on the upper surface. It is believed that recognition of the punctate condition may be overlooked macroscopically and sometimes microscopically due to the age of the gland. Under the microscope this small organ is observed either when a droplet of secretion has formed, as an atomiferous or sessile gland, or subsequent to the disappearance of the secretion, as the punctate condition. Thus it depends on the maturity of the gland. An examination of plants grown in the greenhouse indicated this. On leaves of young seedling plants of fourteen species studied, although the gland cells were distinguishable with the microscope, no "holes" were seen in the leaves or leaf preparations. Preparations from herbarium specimens of the same species were recorded as punctate.

The leaves of most species of *Brickellia* are comparatively thin and have some trichomes along the margin. When the cleared preparation of the most fleshy-leaved of all species, *B. glutinosa*, was examined, the entire leaf margin appeared as a succession of minute depressions and rises. In the former, from the sunken epidermal layer arose small familiar biseriate structures, the glandular trichomes (fig. 85, or seen laterally, fig. 86). Thus the two larger appressed cells seen so commonly on the leaf surfaces are explained by the two uppermost and largest cells of the series and we find the interpretation of the two smaller "bodies" in the smaller basal cells.

The earliest figures found to which these are comparable were those by Solereder (1899, figs. 103 G, H) of *Mikania pubescens* Nutt. and *Chrysanthemum cinerarifolium* Vis., after Vogl. The only bibliographical reference to this author included, does not pertain to those genera. The original figures have not been found. While the exact stage of development to which the figures apply is therefore not known, they might represent the depressed condition. In Solereder's text they are given as illustrations for short-stalked glandular hairs with a head consisting of two rows of cells, common to a number of genera of the *Compositae.* Among the latter are included those listed by Vuillemin (1884) as having biseriate glandular hairs. That adjective has been adopted for the depressed gland as well as the glandular trichome described for *Brickellia.*

It is certain from a comparison of figures given by other writers that while this type is common to very many genera they may vary in stages of development and final form. Steps shown by Rosenthaler and Stadler (1908) for a gland from the surface of a leaf of *Cnicus benedictus* are similar to those followed in *Brickellia,* yet the figure (24 IV Pl. III) of the mature stage has a greater diameter for its height than any seen in this genus. Those authors also include a more dissimilar figure (24 IV Pl. III) from the flowers, stating that it had a more hair-like form than on the leaf. Allowing for variation in height, a remarkable constancy was found in its form in *Brickellia.* The figure most closely resembling the longer biseriate trichome that has been found is of a stalked gland given by Hoffmann (1898, fig. 56B) in his introduction to the *Compositae* as occurring on the petiole of a *Eupatorium.* A general discussion of the terminology pertaining to the forms in all families can be found in Netolitzky (1932).

It is obvious since leaves of the same species can show glandular punctation either conspicuously or not at all, that macroscopic observations of specimens would seem to vary. Robinson stated in his introduction that such punctation was normal in the leaves of the genus but was not always visible. It should also help to clarify matters if it is understood that in *Brickellia* biseriate glandular trichomes are outgrowths of the depressed glands which have been called sessile or atomiferous glands. These are the trichomes which are responsible for the so-called glandular pubescence. From the species cited, this probably holds for glandular puberulence as well, although some smaller glandular uniseriate, capitate trichomes may contribute an additional effect to a shorter indumentum. These were found very commonly especially along the veins of many species (fig. 87). They are small and have not been recognized macroscopically as they never attain the size of those on stalks of biseriate cells. The figure has been drawn from *B. argyrolepis* although it could have been drawn from a large number of species including those where the larger glandular trichomes prevail. There are slight variations from the globular shape to a more ovate terminal cell (*B. Coulteri*, fig. 88). The latter may be intermingled with the former. Similarly, they may vary in length of stalk to about six narrow cells. They distinctly come from one rather than twin cells and bear no relation to the depressed glands.

Observations from these leaf studies have been condensed in the form of table III. Since the species studied karyologically showed most of the distinctive types of trichomes, only those have been included. In the table no attempt was made to record the abundance in each category. Furthermore the condition recorded represents that of one or at most several specimens of any species.

It was found that where the biseriate glandular trichomes make up the pubescence either completely or with only a very few non-glandular trichomes, depressed glands are found rarely if at all (e. g., *B. floribunda* and *B. Wislizeni*). Conversely, species with extraordinarily punctate leaves or numerous depressed glands frequently seem to lack or have very small ones of this type very sparingly along the veins (e. g., *B. cuspidata* and *B. monocephala*). Occasionally such a trichome was found exceedingly rarely along the lower midrib as for instance in *B. scoparia* and *B. laciniata* where only one could be recorded after examination of three different specimens. Such observations emphasize the interrelationship of the sessile glands and this type of glandular trichome in *Brickellia.*

Since depressed glands in the leaves are characteristic of this genus and glandular trichomes are merely an expression of their outgrowth, it is not surprising perhaps that no correlations between these organs and specific karyotypes have been found so far in this study. If more representatives of the *Brachiatae,* which seemed to lack, or show the glands more sparingly, were available, it would be very interesting to see if they would resemble the karyotype with satellites of *B. Coulteri.* The specimen studied cytologically was one of the nineteen on which no depressed glands were found without microscopic examination.

Several instances were found of correlation of the karyotype and the types of trichomes which are non-glandular.

1. This is especially noticeable in the *Baccharideae* where the karyotype consisted of shorter chromosomes. Although *B. laciniata* is almost glabrous and the trichomes are very few and very short, yet they were surprisingly moniliform like *B. californica* and *B. desertorum.* Those of *B. veronicaefolia* may be a little more distinctive in the tendency towards a cap cell, but it must be noticed that Robinson called these moniliform, likely due to their general contour.

2. Significant is the comparison of *B. Palmeri,* which in trichome as in chromosomal complement does not fit with the rest of the *Baccharideae.* The trichome is somewhat transitional and fits better with *B. glomerata.*

3. In the short trichomes of *B. Rusbyi,* there is similarity of type to those of *B. californica, B. desertorum* and *B. laciniata* as there is also in the karyotype.

4. *B. betonicaefolia* and *B. amplexicaulis* tie in with *B. Wislizeni* in having attenuate trichomes, despite the difference in size of heads.

5. *B. incana* is the most unusual, as it is in its karyotype of short chromosomes with one pair of satellites.

6. *B. lanata* has trichomes which, though longer, appear to be drawn-out long acuminate ones, and more similar to some species of the *Reticulatae* than to the long non-septate one of *B. incana,* next to which it had been placed.

TABLE III.—Types of Trichomes

	Species	Non-glandular Trichomes Uniseriate	Glandular Trichomes		Depressed Glands and Punctation in Epidermis	
			Biseriate	Uniseriate, Capitate	Upper	Lower
Section I	*B. diffusa*	Short attenuate				×
Section VII						
Subsection						
1. Clavigera	*B. scoparia*	Scabroid	Rare and short		×	×
3. Microphyllae	*B. microphylla*	Acuminate	Very short to longer ones			
	B. scabra	Scabroid	Very short ones		×	×
	B. Nevinii	Branched		×	×	×
4. Parvulae	*B. dentata*	"dentata"	Very short	×	×	×
	B. brachyphylla	Very short attenuate		×	×	×
5. Reticulatae	*B. venosa*	Long acuminate, towards cap		×	×	×
	B. oliganthes	Long acuminate, towards cap		×	×	×
	B. reticulata	Long acuminate, towards cap		×		×
	B. verbenacea	Long acuminate, towards cap		×		×
6. Amplexicaules	*B. cuspidata*	Short attenuate		×	×	×
	B. betonicaefolia	Attenuate		×		×
	B. amplexicaulis	Attenuate	×	×		
7. Brachiatae	*B. Coulteri*	Attenuate	×	×		×
	B. megaphylla	Long acuminate, towards cap		×		×
8. Baccharideae	*B. laciniata*	Moniliform	Rare and short	×		×
	B. desertorum	Moniliform		×	×	×
	B. californica	Moniliform		×		×
	B. veronicaefolia					
	var. *veronicaefolia*	Towards cap	×	×		×
	var. *umbratilis*	Towards cap	×	×		×
	var. *senilis*	Towards cap		×		×
	B. Palmeri					
	var. *amphothrix*	Long acuminate		×		×
9. Coleosanthus	*B. Rusbyi*	Moniliform		×		×
	B. glomerata	Attenuate toward cap		×	×	×
	B. paniculata	Long acuminate	×	×		×
	B. secundiflora					
	var. *secundiflora*	Attenuate to long acuminate		×		×
	B. tomentella	Attenuate to long acuminate		×		×
	B. nutanticeps	Attenuate		×		×
	B. pendula	Attenuate		×		×
	B. argyrolepis	Attenuate to long acuminate		×		×
	B. adenocarpa					
	var. *adenocarpa*	Long acuminate		×	×	×
	var. *glandulipes*	Long acuminate	×	×		
	B. pacayensis	(lacking)	×			
	B. floribunda	(lacking)	×			
Section VIII	*B. oblongifolia*	Attenuate	×		×	×
	B. Wislizeni	Attenuate	×	×		
	B. Greenei	(lacking)	×			
	B. macromera	Attenuate to moniliform	×		×	×
	B. peninsularis	Long acuminate		×	×	×
	B. grandiflora	Attenuate		×	×	×
	B. incana	Unseptate			×	×
	B. lanata	Very long acuminate				×
Section IX	*B. monocephala*	Attenuate		×	×	×

Types of trichomes as seen by microscopic examination of cleared leaves from annotated herbarium specimens at the Gray Herbarium.
× indicates character present.

It should be emphasized that where one species of a pair (e. g., *B. cuspidata* and *B. lanata*) with similar karyotypes is either nearly glabrous or puberulent in contrast to pubescent, classification of the short trichome cannot be depended upon for correlation. Generally, there is more similarity in such abbreviated forms, *B. laciniata* being exceptional.

One instance of lack of variation of the karyotype accompanying varying kinds of trichomes was found in the subsection *Microphyllae*. However, examination of the types of trichomes of the four species suggests that they are really not so very different except for the branching in the one.

The epidermal cell pattern was found to be so variable in the leaves of the large number of species examined that many preparations would be necessary to make a worthwhile comparative study. The variability was impressive when seen in the leaves of young plants in the greenhouse. In fourteen species, the cell wall was consistently very undulate in outline. From leaves of these same species, taken from herbarium specimens, none were of the same extremely irregular outline. The shapes varied from slightly undulate to that of a block-like pattern. In some, the cells were more rectangular and in others almost isodiametric. Since, as was stated above, smaller leaves were chosen in order to permit mounting on a microscope slide, it is assumed that the differences in the epidermal pattern may have been due in part to the differences in ages of leaves selected. Without comparable specimens, growing under similar conditions, which would be most difficult in this genus, comparative data on the epidermal cells would be unsatisfactory.

Pappus

Differences in the number of setae of pappus failed to be of any value in classification, Robinson states, for they varied from one species to another from 10 to more than 80. Of their particular nature, less was written. The pappus was given significance in only one sectional heading, that of *Steviastrum* II, with three species, where it was stated to be short but distinctly plumose. Otherwise it is referred to only in *B. brachyphylla* as plumose, and in *B. monocephala* as somewhat plumose.

Lactic acid mounts, similar to those used in a study of *Liatris* hybrids (Gaiser, 1951) were examined, particularly of those species studied karyologically, but with some additional ones to include other sections. Measurements of the length of the barbules showed considerable variation from those recognized by the taxonomist as plumose, which were the longest, to very short in the most delicate found (*B. diffusa*). Also they vary greatly in the number of barbules, being fewer and more distantly spaced on the seta in some and numerous and closely crowded on others. The variation on this basis is so great as to almost make the arrangement seem different when there are but few. At the tips of the setae, these barbules appear to come from the axis in a somewhat spiral fashion. However, for the main length of the seta, their arrangement is clearly distichous when the barbules are numerous so that they give the appearance of a mid rachis with short slanting pinnules. When there are fewer scattered barbules, the pattern is less regular. A striking exception to this, was noted in the annuals, *B. diffusa* and *B. filipes*, where there is also a median row of barbules, so that it becomes tri- instead of distichous.

As there are further inter-generic variations in the closely related group of the *Kuhniinae*, discussion for the whole group will be presented later (MS in preparation).

Discussion

It has generally been accepted that annuals have been derived from perennials and, among the latter, herbaceous ones from the woody types (Sinnott and Bailey, 1914). Eight perennial species of *Brickellia*, with equal representation of herbaceous and shrubby forms, differ markedly in their shorter chromosomes from twenty-five others with longer ones. Since the one annual of the genus in which the chromosomes have been studied has a karyotype of short chromosomes, it seems reasonable to assume that in this genus evolution has gone on generally in line with a reduction in chromatin mass. Delaunay (1926) was the first to postulate that reduction in chromosome size accompanied evolutionary advancement. In the genus *Crepis* of the *Cichoreae* (*Compositae*), the thorough studies of Babcock (1947) and a great number of his fellow workers, have shown a reduction in the number of chromosomes as well as of the size.

By this reduction hypothesis, the species with the greatest amount of chromatin would be considered the most primitive.

From the present study, this condition is found in *B. monocephala*. This species stands at the opposite extreme from *B. diffusa*, the two species appearing as the first and the ninety-first, or last, in Robinson's treatment of the genus. *B. monocephala* is a singular species with only a solitary terminal head, borne on a long, naked peduncle. It consists of well over one hundred florets, in contrast to the slender heads of about eight flowers in a large loose panicle, of *B. diffusa*. The phyllaries are broadest and the only ones in the genus to have markedly scarious margins. The achenes of the former are the third largest of the genus. Underground it has a short fleshy rhizome with one or two annual buds, as seen in a fresh specimen received from Mexico, in contrast to the fibrous root system of the annual and of many of the other perennial species. It is known from very limited localities, as the hills of El Salto in Mexico state where it borders on Hidalgo, the environs of Morélia, and one collection from Durango. The species, *B. hymenochlaena* Gray, with a similar knobby root, has about a dozen heads, approximately 40-flowered, borne somewhat as an umbel on one stem. It also is rare[10] and found only in the mountains of San Luis Potosi, Hidalgo, Puebla and Oaxaca. Another species, *B. simplex* Gray, which is rather intermediate between these two, since it occurs either with single large heads or a few of about sixty flowers on one long peduncle, seems to be a related species of the northern Mexican States, Chihuahua, Sonora, and adjoining Arizona. Though these two species were not represented in this cytological study, we believe that the next most nearly related species is *B. grandiflora*, which has a long fusiform root and heads of 20–38 flowers. In the general section on root, rootstock and caudex of his introduction, Robinson singled out these species as having a form exceptional to the prevailingly slender fibrous one. The four are very similar in having petiolate, deltoid to ovate leaves, as well. *B. grandiflora* was found to have a karyotype approximating the size of that of *B. monocephala*. The former is one of the species with the widest range in the United States where it reaches the northern limits of the genus. Thus, it has more successfully adapted itself than the other three. With the largest chromosomes found in *B. monocephala*, and probably the

[10] In conversation with the late Prof. Conzatti, he stated this species was very difficult to find.

next in *B. grandiflora*, it is possible that these represent a line of specialization in root-form from a primitive group and of them the nearest to the ancestral type may well be *B. monocephala.*

While these are herbaceous perennials, among the shrubby species, at least *B. argyrolepis*, *B. pacayensis* and *B. adenocarpa*, having heads of 25–30 flowers, had chromosomes almost if not equally as long as *B. grandiflora*, apparently also lacking two of the short class. It is not possible to say whether the present day herbaceous or shrubby types have evolved more rapidly. It is evident, nevertheless, that among the shrubby ones, and possibly in the woodiest of the genus, *B. argyrolepis*, appears a karyotype of chromosomes closely approaching what should be the most ancestral according to the hypothesis of reduction. That group, however, suggests by the form of root, as well as the more perishable above-ground parts, a specialization which may have started off from a shrubby ancestor unlike any we have today. As phylogenetic changes rarely have been found to move in one straight line, it is readily understandable, too, that in the woody forms the heads might be more reduced. There is at least good correlation between taxonomic characters and chromosome size moving in unison in evolutionary changes in *Brickellia.* At the one extreme occurs an annual with short chromosomes and at the other a perennial with the longest chromosomes, and next to it related perennials and woody shrubs with chromosomes of approaching length. That in between a minority of shrubs, and perennials also, have short chromosomes, may perhaps show the way.

As yet no direct evidence has been obtained which would explain the amount of shortening of chromosomes from the general size as in those of a species like *B. californica.* Some indications of how slight reduction of chromatin content might occur, were seen among the preparations studied. Twelve accessions of this species showed an unvarying karyotype of short chromosomes. However, in one other, from the Chiricahua Mountains of Arizona, a terminal body was frequently seen on one of the longest chromosomes. Although this was not a regular feature of the species, it may be of that population. If that segment of the chromosome were heterochromatic and lacking in genes of particular significance, it might become separated and lost